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T-Cell Receptor Vα Spectratype Analysis of a CD4-Mediated T-Cell Response against Minor Histocompatibility Antigens Involved in Severe Graft-versus-Host Disease

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ABSTRACT

Although $CD4^+$ T cells can have an important role in mediating lethal graft-versus-host disease (GVHD) directed to multiple minor histocompatibility antigens (miHA) after bone marrow transplantation, their precise characterization and effector function remains elusive. In this regard, T cell receptor (TCR) Vβ spectratype analysis has been a powerful tool for identifying donor $CD4^+$ T cell populations expanding to host miHA after bone marrow transplantation in the major histocompatibility complex–matched C57BL/6 (B6) → C.B10-$H2^b$ (BALB.B) model of lethal GVHD. Removal of all of the $V\beta^+$ T cell families containing these responding cells from the donor inoculum has proven to be an effective means of preventing the development of GVHD. Previous studies have also found that of the 11 miHA-responsive B6 $CD4^+$ $V\beta^+$ T cell families, transplantation of $V\beta2^+$ and $V\beta11^+$ T cells together into lethally irradiated BALB.B mice appeared to be primarily responsible for the severity of resultant GVHD. Further focusing on these critical CD4 responses, in this study we demonstrate that B6 $CD4^+V\beta11^+$ T cells alone can induce lethal GVHD in BALB.B recipients. In addition, immunohistochemical staining of host lingual and intestinal epithelial tissues supported the capacity of $V\beta11^+$ T cells to infiltrate typical GVHD-associated target areas. To further characterize the specific $CD4^+V\beta11^+$ T cells involved in this anti-miHA response, TCR Vα spectratype analysis was performed and indicated that 6 Vα chains were used by this reactive population. These results provide further evidence that a restricted repertoire of T cell specificities, presumably recognizing a correspondingly low number of miHA, is sufficient for the induction of severe GVHD.

KEY WORDS

T cell receptor • V alpha chain • Graft-versus-host disease • Repertoire • Minor histocompatibility antigens

INTRODUCTION

The wide diversity of the T cell repertoire is achieved by both rearranging gene segments composing each T cell receptor (TCR) α and β chain and combining these 2 chains to form the final heterodimer structure. During a T cell response, the repertoire can become significantly skewed as a result of the significant clonal and/or oligoclonal expansion of antigen-specific T cells [1-3]. This same repertoire skewing can also be observed in the context of alloreactive responses, as in the case of the development of graft-versus-host disease (GVHD) after allogeneic blood and marrow transplantation [4-7].

GVHD is mediated by T cells from the donor hematopoietic stem cell inoculum and is often characterized by immunopathological injury to the skin, intestinal tract, and liver, leading to morbidity and mortality [8]. Although it is well accepted that remov-

ing donor T cells from the graft can significantly decrease the incidence and severity of GVHD, the lack of a functioning T cell repertoire in the recipient results in increased leukemic relapse, engraftment failure, and opportunistic infections [9]. An alternative approach is to identify and remove only those specific T cells primarily involved in the alloreactive antihost response associated with GVHD, thus preserving a large part of the T cell repertoire to provide the beneficial T cell–associated effects. In this regard, we have previously used TCRVβ CDR3-size spectratype analysis to characterize the skewing of the T cell repertoire in response to minor histocompatibility antigens (miHA) in the B6 → BALB.B $H2^b$-matched strain combination [10-12]. Using the information obtained from this analysis to manipulate the donor inoculum by depleting the alloreactive $Vβ^+$ families, we found improved survival rates for the transplantation recipients [5,10-12]. Similarly, in the haploidentical B6 → (B6xDBA2)F_1 strain combination, spectratype analysis was also used to identify leukemia-reactive $Vβ^+$ T cells, which when transplanted into lethally irradiated tumor-challenged mice could mediate an effective graft-versus-leukemia response without GVHD development [4,5,10,13,14].

An appreciation for the role of the TCRα chain in terms of antigen recognition is evolving. Several studies using single α or β chain transgenic mice indicated that most responding T cells could use various β chains but only 1 α chain [15-18]. These murine studies, indicating a contributing role of the TCRα chain in antigen recognition, were further supported by analysis of lesions in autoimmune- and GVHD-associated patients [6,17,19,20]. In addition, crystal structure analysis of a TCR interacting with a peptide–major histocompatibility complex (MHC) II complex revealed that the α chain actually made more peptide contacts than the β chain [21]. Other studies showed a requirement for conservation within both α and β chains when mutations within the J regions could eliminate T cell responses to peptide [22]. Taken together, these results suggested that the TCRα chain may play a more significant role in antigen recognition than was originally thought. Consequently, we hypothesized that analysis of a specific $CD4^+$ T cell response against miHA during the development of GVHD would reveal preferential Vα chain usage.

To investigate the breadth of TCRα chain diversity, we again used the B6 → BALB.B strain combination and focused on 1 of the 2 $CD4^+Vββ^+$ T cell populations that were previously observed to be directly involved in the severity of the disease [4]. Spectratype analysis indicated that B6 $CD4^+$ $Vβ2^+$ and $Vβ11^+$ T cells were skewed in the antihost response, and that transplantation of an enriched population of both of these cells into lethally irradiated BALB.B recipients resulted in the development of lethal GVHD, whereas the transplantation of the remaining $Vβ2^-$ and $Vβ11^-$ T cells did not [12]. Consequently, in the current study, we sought to demonstrate the capacity of B6 $CD4^+$ $Vβ11^+$ T cells on their own to induce GVHD, as well as to examine the complexity of this response by TCR Vα spectratype analysis. The results indicated that B6 $CD4^+$ $Vβ11^+$ T cells could be found infiltrating both lingual and intestinal sites of early immunopathological injury associated with GVHD. Furthermore, these reactive T cells used only 6 Vα chains, further demonstrating that an allogeneic response of limited scope can be of profound consequence in the development of GVHD.

MATERIALS AND METHODS

Mice

C57BL/6By (B6; $H2^b$) and C.B10/LiMcdJ (BALB.B; $H2^b$) mice were purchased from the Jackson Laboratory (Bar Harbor, ME). In addition, BALB.B mice were provided from our own breeding colony. Male mice, age 6-12 weeks, were always used as donors; both female and male mice, age 8-12 weeks, were used as recipients. Mice were housed in a pathogen-free environment in autoclaved microisolator cages and were provided with autoclaved food and water ad libitum. The treatment of the animals was approved by the Institutional Animal Care and Use Committees of both Thomas Jefferson University and Hackensack University Medical Center.

Isolation of $CD4^+$ T Cells and GVHD Induction

BALB.B splenocytes (2×10^7) were injected intraperitoneally into B6 donor mice 17-21 days before GVHD induction. Spleens and lymph nodes (LNs) were collected from the presensitized B6 mice, resuspended in Gey's balanced salt solution, containing 0.7% NH_4Cl, to eliminate erythrocytes from the suspension, and washed with phosphate- buffered saline (PBS; BioWhittaker, Walkerville, MI) containing 0.1% bovine serum albumin (BSA; Sigma, St. Louis, MO). B cells were then depleted from the spleen and LN cells by a panning procedure with plate-bound goat anti-mouse IgG (1:200 dilution; Cappel-Organon, Teknika, West Chester, PA) for 1 hour at 4°C. Adherent cells were washed and resuspended in PBS containing 0.1% BSA (PBS/BSA) along with rat IgM anti-CD8 monoclonal antibody (mAb; 1:100 dilution of supernatants from hybridoma cells grown in CelLine flasks, clone 3.168; BD PharMingen, San Diego, CA [23]) and guinea pig complement (1:6 dilution; Rockland, Boyertown, PA) for 45 minutes at 37°C to deplete $CD8^+$ T cells. Bone marrow cells were obtained from primed B6 mice by flushing femurs with PBS/BSA, and T cell–depleted bone marrow cells